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Art. II.—*On Bitter Pit and the Sensitivity of Apples to Poison.*

By ALFRED J. EWART, D.Sc., Ph.D.
(Professor of Botany and Plant Physiology in the Melbourne University).

[2nd Paper.]

(With Plates III.–V.).

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In the first paper[1] a detailed account of the action of various poisons on the pulp cells of apples was given, and their extreme sensitivity to certain metallic poisons shown. This sensitivity was so great that it was possible to produce pitting in apples by quanties of lead, mercury and copper so minute as to be incapable of detection, even by delicate methods of technical analysis, at least with the quantities of material available.

Many points still remained open, however, both in regard to the influence of external conditions upon the sensitivity of apples to poison, and also in regard to the bearing of the facts observed upon the problem of bitter pit. In addition, Mr. McAlpine has recently published a voluminous report on bitter pit, in which he confidently assumes that bitter pit and poisoning have no relationship.

The influence of temperature on poisoning.

It has long been known that at low temperatures or when in cool storage, the development of bitter pit is retarded, and Scott (Phytopathology, 1911, p. 32) found the same to be the case with the development of the spots, which he concluded were due to spraying with arsenate of lead. Hence it was of interest to determine the influence of temperature upon the formation of pits by direct poisoning. The method used was as previously described. Apples were floated each in 1 litre of the poisonous solution after removing fragments of the cuticle of approximately a square millimetre in area, from points 1 or more centimetres apart around the periphery of the apple. (See table I.)

1 Proc. Roy. Soc. Victoria, vol. xxiv., 1912, p. 367.

TABLE I. MERCURIC CHLORIDE. STATESMAN APPLES.

Strength of Solution.	At 25°C.—26°C.		At 10°C.—11°C.		At 0°C. 1°C.	
	After 2½ days' immersal.	After 3½ days in air.	After 3½ days' immersal.	After 3½ days in air.	After 3½ days' immersal.	After 3½ days in air.
1 per 10,000	Brown areas 8–12 mm. diameter nearly confluent.	Pits coalesced to ring of dead tissue 1–3 mm. broad and 2–10 mm. deep.	Spots[1] pale brown 3–6 mm. diameter.	Pits 5–10 mm. diameter and 2–5 mm. deep.	Pale brown spots 1–2 mm. diameter.	Pits 3–5 mm. diameter and 1-2 mm. deep.
1 per 100,000	Pale brown areas 3–5 mm. diameter.	Pits 4–10 mm. diameter and 2–5 mm. deep.	Spots very pale and 1–2 mm. diameter.	Pits 2–3 mm. diameter and 1–2 mm. deep.	Very faint superficial browning.	Pits 1–2 mm. diameter and 1 mm. deep.
1 per 1,000,000	Very pale spots 1–2 mm. diameter	Pits 3–5 mm. diameter and 2–3 mm. deep	Faint browning on shaded side only 1mm. diameter	Superficial browning to pits 1 mm. diameter and depth.	No signs of poisoning	No signs of poisoning.
1 per 10,000,000	No distinct signs of poisoning.	From superficial browning to pits 2 mm. diameter and depth.	No distinct signs of poisoning.	From faint superficial browning to no signs of poisoning.	No signs of poisoning	No signs of poisoning.

1] The largest spots are usually on the paler shaded side, which appears to be more sensitive to poison.

TABLE II. COPPER SULPHATE. YATES PIPPIN.

Strength of Solution.	At 28°C.–30°C. After 3½ days' immersal.	At 28°C.–30°C. 3½ days in air.	At 12°C.–13°C. After 3½ days' immersal.	At 12°C.–13°C. 3½ days in air.	At 0°C.–1°C. After 3½ days' immersal.	At 0°C.–1°C. 3½ days in air.
1 per 1,000,000	No signs of poisoning except barely perceptible rim to some of prepared spots.	Slight to distinct superficial browning on prepared spots.	No signs of poisoning.	Superficial browning only.	No signs of poisoning.	No signs of poisoning.
1 per 100,000	Pale brown areas 2–4 mm. diameter to each prepared spot.	Pits 2–4 mm. diameter and 2–4 mm. deep.	Dark rims to each spot, 2 mm. diameter.	Pits 1–2 mm. diameter and ½–1 mm. deep.	Faint brown rims to some but not all of prepared spots.	Slight to no superficial browning.
1 per 10,000	Dark brown to black areas 2–4 mm. diameter.	Same as with 1 per 100,000, but pits darker.	Dark rims to each prepared spot, 2–3 mm. diameter.	Pits 1–3 mm. diameter and ½–1½ mm. deep.	Pale brown rims to all prepared spots, none exceeding 1 mm. diameter.	Superficial browning only.
1 per 1000	Dark brown to black areas, 2–5 mm. diameter.	Pits fused to a curved band of dead tissue 1cm. deep.	Black areas to each prepared spot 2–3 mm. diameter.	Pits 2–3 mm. diameter and 1–2 mm. deep.	Brown rims to all prepared spots none exceeding 1 mm. diameter.	Pits 1–2 mm. diameter and superficial to 1 mm. deep.

Prepared spots 1½ cm. apart.

With Statesman apples therefore, the mercuric chloride ceases to exert any poisonous action at 0—1 deg. C. in a concentration of 1 per million, but continues to show a poisonous action at 25—26 deg. C. in a concentration of 1 in 10 millions. Comparing all the results, we may say that a fall of temperature of 25 deg. C. lowers the poisonous concentration 10 to 100 times.

Using copper sulphate and Yates' Pippin apples, the results were obtained as given in Table II.

In this case copper sulphate in a concentration of 1 in 10,000 exercised about the same poisonous action at 0—1 deg. C., as a concentration of 1 per 1,000,000 did at 28—30 deg. C., and the latter concentration exercised no poisonous action at all at the lower temperature. Under the conditions of the experiment therefore, copper sulphate was a hundred times as poisonous to the pulp cells of apples at 28—30 deg. C., as it was at 0—1 deg. C.

The influence of diffusion.

Temperature exercises a most important influence upon the rate of diffusion, and hence also upon the rate at which the poison in solution would diffuse to the prepared spots on the apples tested The rate at which the molecules of the dissolved substance reach the receptive surface is an important factor in determining the concentration at which a poisonous action can be exercised.

Thus in the case of Yates' Pippin apples floated on mercuric chloride solution at 15 deg. C. for three days, the poisonous limit was reached with a concentration of one gram in 10 million cubic centimetres of water. On the other hand, if the poisonous solution was allowed to trickle directly over three prepared spots, three times daily for three days, from a fine tube at a temperature of 14—15 deg. C., a slight poisonous action was still shown with a concentration of 1 gram in 1000,000,000 c.c of water.

Yates' Pippin. Mercuric chloride. 1 litre of solution run over three prepared spots, three times in three days. Temperature 14—15 deg. C. Trickled directly over prepared spots from fine tube.

1 per 100,000,000	-	No distinct signs of poisoning, but after 1 week in air, superficial browning with blacker rim to each prepared spot.
1 per 100,000,000,000	-	No signs of poisoning, and after one week in air no distinct signs of poisoning as compared with control treated with pure distilled water.

The same experiment as above was repeated, but the prepared spots were surrounded by a square centimetre paraffin cell 2—3 mm. deep through which solution trickled (see Pl. 3), 1 litre was used and trickled once daily through the cells and over three prepared spots for seven days.

1 per 100,000,000	Slight superficial browning	After 7 days in air superficial browning extending up to ½ mm. deep.
1 per 1,000,000,000	No distinct signs of poisoning	Faint to distinct superficial browning.
1 per 10,000,000,000	No signs of poisoning	No signs of poisoning.

Even superficial browning under the microscope can be seen to affect several successive layers of cells, one below the other, and even in small pits the number of cells affected soon runs up to the thousands. Since it must take a definite number of molecules even of mercuric chloride to completely poison each cell, there must be a limit to the dilution at which a perceptible poisonous action can be exercised.

A litre of a 1 per 10,000,000,000 solution contains $\frac{1}{10,000}$ of a milligram of mercuric chloride, representing some $\frac{10^{15}}{3}$ individual molecules, and assuming that 1000 cells are exposed to the absorption of poison, and that under the condition of the experiment at least $\frac{1}{300}$ to $\frac{1}{1000}$ of the molecules present are absorbed, then it would require at least 10^9 (one thousand million) molecules of mercuric chloride to poison a single pulp cell of an apple such as Yates' Pippin, which is one of the most resistant varieties. It is possible that a lesser number of molecules, say, 10 to 100 millions, might be able to arrest diastatic activity in a pulp cell without necessarily killing it.

To obtain some idea as to the influence of diffusion and of convection currents upon the conveyance of poison, comparative experiments were performed with apples on water, and on melted 10 per cent. gelatine allowed to set after adding poison. A preliminary test showed that ordinary gelatine contains traces of poisonous materials, but when well washed these are reduced to a mere trace. The apples must of course be clean, and the gelatine sterilised by two steam heatings, since fungi are able to develop on solutions poisonous to apples.

PREPARED YATES PIPPIN. ALL AT 12-13°C. FOR 1 WEEK IN 200 c.c. MERCURIC CHLORIDE.

	1 per 100,000.	1 per 1,000,000	1 per 10,000,000	No $HgCl_2$
In watery solution.	Pits 2-5 mm. diameter and 2-3 mm. deep.	Pits 2-3 mm. diameter and 1-3 mm. deep.	From faint superficial browning to pits 1 mm. diameter and depth.	No signs of poisoning.
In well washed and sterilized 10% gelatine.	Slight superficial browning to pits 1 mm. diameter and depth.	From no signs of poisoning to slight superficial browning.	From no signs of poisoning to slight superficial browning.	From no signs of poisoning to slight superficial browning.

At first sight this experiment would seem to show that the conveyance of poisons to the prepared spots took place mainly by convection and mixing movements, although the cylinders containing liquid were not disturbed, and were kept free from vibrations, and kept at as uniform a temperature as possible. Mercuric chloride, however, coagulates gelatine in the presence of sodium chloride, and although no salt was present, the mercury evidently enters into combination with the gelatine, just as mercuric nitrate will precipitate gelatine by itself, so that the diffusion of the mercury is either stopped or very greatly retarded.

Hence to obtain a true diffusion comparison, agar and sulphuric acid were used. These do not enter into combination; the sulphuric acid diffuses as rapidly through agar as in stationary water, and the agar when well washed exercises no poisonous action on the pulp cells of prepared apples, while it has also the advantage of standing high temperatures better without liquefying.

Both the water cylinders and the agar cylinders were kept free from disturbance or vibration, the temperatures were kept as uniform as possible, the apples were picked Yates' Pippins all 15 centimetres diameter, and each with 15 prepared spots of 1 square millimetre area, equidistant around the periphery. The 1 per 10,000 solution contained 1.8 grams of pure concentrated sulphric acid to 9999 cubic centimetres of water, or to 9999 cc. of $1\frac{1}{2}$ per cent. agar solution; 150 cc. being used to each apple.

YATES PIPPIN AND SULPHURIC ACID.

Exposure and Temperature	Medium. Water.	Agar.	Total bulk of poisoned tissue	Rates of diffusion of H_2SO_4
3 days at 0°C–1°C	Superficial browning to pits 1–3 mm. diameter, and 1–2 mm. deep	Superficial browning to pits 1–2 mm. diameter and depth	0.002 c.c.	1 at 0°C.
3 days at 10°C–11°C	Pits 2–5 mm. diameter and 2–3 mm. deep	Pits 1–3 mm. diameter and 1–2 mm. deep	0.003 c.c.	1.53 at 10°C.
3 days at 29°C–30°C	Pits partly confluent and 5–10 mm. deep	Pits partly confluent and 5–8 mm. deep	11.25 c.c.	3.83 at 29°C.

Hence it follows that in still water the poison reaches the prepared areas mainly by diffusion, and hence the poisonous action is only slightly decreased when the acid reaches the apple by diffusion alone through agar. In three days only a small portion of the acid present could reach the prepared areas by diffusion, so that the sensitivity of the pulp cells to sulphuric acid is fairly pronounced.

Furthermore, the comparison between the rates of diffusion and the bulk of poisoned tissue at different temperatures, shows clearly that the increased poisonous action at the higher temperatures is not merely a matter of diffusion, but is mainly due to the inherent sensitivity to poison being greater at the higher temperature.

The same is shown by the following test, in which three experiments were started at three preliminary temperatures (nine in all), and of each set of three, one was subsequently kept in air for four days at 0—1 deg. C., one at 10—11 deg. C., and one at 30 deg. C. During the preliminary immersal for three days, the apples were all in a 1 per million solution of mercuric chloride, so that the prepared spots in each set of three received the same amount of poison by diffusion, but during the subsequent exposures in air a consistently greater poisonous action was exercised at the higher temperatures.

YATES PIPPIN IN 1 PER MILLION $HgCl_2$

Preliminary treatment.	Subsequent treatment for 4 days in air.		
Immersed for:	0°C.–1°C.	10°C.–11°C.	30°C.
3 days at 0°C–1°C. From very faint superficial browning to no signs of poisoning	Superficial browning only	Distinct but superficial browning on each prepared spot	Pits 1-2 mm. diameter superficial to 1 mm. deep.

Preliminary treatment. Immersed for:	Subsequent treatment for 4 days in air. 0°C.-1°C.	10°C.-11°C.	30°C.
3 days at 10°C.–11°C. Faint to no superficial browning	From superficial browning to pits 1–2 mm. diameter and 1 mm. deep	Pit 1–2 mm. diameter and 1 mm. deep	Pits 1–3 mm. diameter and 1–2 mm. deep
3 days at 30°C. Brown spots 2–4 mm. diameter	Pits 3–5 mm. diameter and depth. Pit tissue pale brown, unshrivelled and containing some living plasmolysable cells	Pits 3–10 mm. diameter and 5–6 mm. deep, and broader below surface than on skin. Tissue brown and partly shrivelled	Pits 6–10 mm. diameter and 6–8 mm. depth, partly confluent. Tissue dark brown and shrivelled

The influence of temperature upon the sensitivity to poison is shown also by non-metallic poisons. Thus chloroform poured over the surface and then allowed to evaporate, produced brown pits over the surface below the lenticels, and with a watery solution the following results were obtained :—

Yates' Pippin. Chloroform. All showing no distinct superficial signs of poisoning after three days' immersal. Then all at room temperature for four days and examined.

Concentration.	Temperatures during Immersal. 30°C.	11°-12°C.	0°-1°C.
1 per 1000 c.c. (shaken up and excess allowed to slowly dissolve)	Pits superficial to 2 mm. diameter and depth.	Faint to no superficial poisoning.	No distinct signs of poisoning
1 per 10,000 c.c.	Pits superficial to 1 mm. deep.	No signs of poisoning.	No signs of poisoning
1 per 100,000 c.c.	No distinct signs of poisoning.	No signs of poisoning.	No signs of poisoning

Thus a solution of chloroform capable of exercising a distinct poisonous action at 30 deg. C., was non-poisonous at 0 to 1 deg. C. The presence of chloroform appeared to retard the browning of the affected cells, and Yates' Pippin appears to be 10 to 100 times as resistant to chloroform as Jonathans. (1st Paper, p. 402).

In another experiment normal apples were immersed in pure chloroform for short periods of time, and then kept in air for a week at the temperatures given.

Yates' Pippin. Normal surface. Soaked in chloroform, and then kept in air for 1 week at 14—15 deg. C., and at 0–1 deg. C. The calyx and stalk ends closed with paraffin.

Temperature	Time of Immersal in Chloroform. 10 seconds	100 seconds	1000 seconds
0°C–1°C.	Several shallow pits up to 5mm. diameter and 1 mm. depth.	As before but pits slightly more numerous.	General browning over whole surface from ¼ to 2 mm. deep.
14°C–15°C.	Several sunken pits up to 8mm. diameter and 2 mm. total depth.	Numerous, larger and more deeply sunken pits up to 3 mm. depth, partly confluent.	General browning over whole surface from 2 to 8 mm. deep.

In this case the same amount of poison enters in each pair of apples subsequently kept at different temperatures, but the poisoning action is less at the lower than at the higher temperature.

he poisonous action of cell contents and cell products.

It has been suggested that the escape of the cell contents either by bursting or by exudation might cause the poisoning of neighbouring cells and the formation of bitter pit. Against this is the fact that young apples may be punctured without any result beyond the production of a superficial scar or depression when adult, but it seemed advisable to test the influence of the expressed sap and of different cell products on living pulp cells.

The influence of expressed sap on prepared apples.

The sap was rapidly expressed by pressure, and sound prepared apples floated in pure sap, in 20 cc. of sap to 80 water, and in 2 cc. of sap to 98 water for two days. They were immediately examined and also after five days in air, but no signs of poisoning was shown on any of the prepared spots, either using Yates' sap on Yates' apple, Sturmer sap on Sturmer apple, or Yates' sap on Sturmer apple, or Sturmer sap on Yates' apple.

After two days the liquid develops micro-organisms, and after three-four days' immersal, superficial browning may be shown. If the sap is boiled, sterilised vessels used, and the apples coated with paraffin before preparing for immersal, the expressed sap may remain practically sterile for three or four days, and no signs of poisoning were then shown in this time on any of the prepared spots. Evidently, therefore, the unaltered sap of apples is not poisonous to the pulp cells when applied to them externally. Apparently the ectoplasmic membrane of the pulp cells has the same diosmotic relationships to the vacuolar contents as the vacuolar

membrane has, so that a dissolved substance which is not poisonous inside the cell is not poisonous outside it. This is the more surprising because some of the cell contents when applied in pure form, are capable of exercising a poisonous action. Possibly this may be a question of ionization, of combination or of relative influence on the surface tension of the ectoplasmic membrane, and hence on diosmosis. The acidity of the sap of the ripe Yates' Pippin apples used was such that 2½ litres were equivalent to 10.6 grams of normal sodium carbonate. This is within the limit of dilution for the poisonous action of malic, citric and oxalic acids when used in pure form, and with exposures of a week's duration. It has already been shown that the poisonous action of a mixture of substances may be much less than when each is applied singly, and apparently it is this fact which explains partly at least, the practically non-poisonous character of freshly extracted apple sap to pulp cells when applied externally.

Alkali.—Since the protoplasm of the pulp cells is alkaline, although the sap is acid, the vacuolar membrane must be able to prevent the acid in the sap from entering the protoplasm, and we should expect to find the protoplasm more resistant to alkalis penetrating from outside than to acid. This has already been shown to be the case with ammonia and Jonathan apples, and it applies still more with caustic potash and the more resistant Yates' Pippin.

CAUSTIC POTASH. PREPARED YATES PIPPIN IMMERSED FOR 5 DAYS AT 3–18°C.

Strength of Solution.	Result.
5 grams per 1000 c.c.	Dark brown pits to each prepared spot, 1–2 mm. diameter and depth.
1 gram per 1000 c.c.	Faint superficial browning to no signs of poisoning.
1 gram per 10,000 c.c.	No distinct signs of poisoning.
1 gram per 100,000 c.c.	No signs of poisoning.

With dilute solutions, however, the CO_2 produced by the respiring pulp cells would suffice to turn the diffusing KHO molecules into potassium carbonate, so that the alkaline action of caustic potash would be less evident than with equal dilutions of ammonia. In fact, with a 1 per 1000 dilution, the poisonous action may be largely due to the potassium ions, rather than to the hydroxyl ions.

Alcohol.—During the anaerobic respiration of apples small quantities of alcohol are produced, and are apparently to some extent transferred from the protoplasm which forms them into the cell

sap, since alcohol is one of those substances which are freely permeable to the protoplasmic membrane.

For the tests pure absolute alcohol was used, prepared Yates' Pippin apples being immersed for four days in the solution and examined after three days in air. The temperature averaged 14—16 deg. C.

1 c.c. of absolute alcohol per 10 c.c. of mixture. (100 c.c. of solution).	- From superficial browning to pits 1–2 mm. diameter and depth.
1 c.c. of absolute alcohol per 100 c.c. of mixture. (1000 c.c. of solution).	- From superficial browning to pits 1–2 mm. diameter and depth.
1 c.c. of absolute alcohol per 1000 c.c. of mixture. (1000 c.c. of solution).	- No signs of poisoning.
1 c.c. of absolute alcohol per 10,000 c.c. of mixture. (1000 c.c. of solution).	- No signs of poisoning.

Hence in its poisonous action on the pulp cells of apples, alcohol comes next to pure water, and is one of the least poisonous of all the substances tested.

Tannic acid or Gallo-tannic acid is present in the pulp both of ripe and unripe apples, and Mr. P. R. Scott's analyses give the amount as usually less than 0.1 gram per cent., and as being slightly more abundant in pitted than in normal apples.

Yates' Pippin prepared. After seven days' immersal at 13—16 deg. C., in tannic acid solutions in water.

1 gram per 100 c.c.	- Brown pits 1–2 mm. diameter and depth.
1 gram per 1000 c.c.	- Brown pits 0.5-1 mm. depth. 1-2 mm. diameter.
1 gram per 10,000 c.c.	- No distinct signs of poisoning.
1 gram per 100,000 c.c.	- No signs of poisoning.
1 gram per 1000,000 c.c.	- No signs of poisoning.

Sturmer Pippin apples were sensitive to tannic acid in ten times greater dilution as compared with Yates' Pippin.

Tannic acid, therefore, comes next to alcohol in the feebleness of its poisonous action, and is less poisonous than many nutrient salts are when applied singly. It may safely be assumed therefore that the slight differences in the percentage of tannic acid supposed to exist between pitted and clean apples have no causative relationship with bitter pit.

The influence of mechanical injuries.

Stewart[1] noted that in a bruised fruit, the injured portion contained an abundance of starch, but not the uninjured portion. This of course would only be the case when the injury was caused at that period of development when the pulp cells are packed with starch. Bruises on quite young fruits, before the starch grains have been deposited, and on adult fruits after they have dissolved, do not show this peculiarity. Varcollier's[2] explanation is that in the bruised cells the tannin inhibits diastatic activity. McAlpine (l.c. page 21) states, "The death of the cells, in my opinion, is quite sufficient to account for the persistence of the starch in the bruised cells," being apparently unaware that diastase will act as well in a non-living medium as in a living cell. The explanation is merely that the escape of the sap from the bruised dead cells removes the medium into which the protoplasm excretes the sugar as it is formed, so that in the protoplasm of the dead cell the percentage of sugar inhibitory to further diastatic action is soon reached. Although diastase is a fairly stable compound when dry, in the moist cell it soon undergoes post-mortem decomposition, and hence the possibility of a post-mortem diastatic action is limited in time.

In any case the presence of starch grains in dead pulp cells, and their absence from the living pulp is not an infallible indication of bitter pit, and indeed this symptom only accompanies bitter pit when the defect begins to develop during the second or "starch" stage of the apple. If bitter pit arises during the early "proteid" stage of the apple or the adult "sugar" stage, the dead cells contain no more starch than the living ones. (1st paper, pp. 410-415.)

The relation of Bitter Pit to vascular tissue.

In those cases where bitter pit is due to poisonous substances absorbed through the roots, it would be natural to expect the dead tissue to be more or less closely associated with the conducting vascular bundles. This was actually observed by Wortmann (Landw. Jahrb. xxi. 1892, p. 663), but his generalisation is too sweeping. The vascular network in apples is comparatively small meshed, so that any large pit or spot must appear to be associated with a vascular bundle. Small pits, however, particularly when late in development, may be found which have no special connection with any one vascular bundle, and larger spots may be sometimes

1 Stewart, F.C., New York State Station, Bull. 164, 1899.

2 Varcollier, G., Compt. Rend. Acad. Sci., 141, p. 405, 1905.

found whose centres lie between two vascular bundles instead of on either.

The vascular system of the apple.

In a paper published by the Linnean Society of New South Wales, vol. xxxvi., 1912, pp. 613-656, D. McAlpine describes the vascular system of the apple (and pear), and apparently considered that its existence was unknown to Sachs and other botanists. As a matter of fact, the vascular systems of the apple and pear, as well as of fruits generally, were first described and figured by Nehemiah Grew in the year 1682 (Anatomy of Plants, Book IV., pp. 179-182, plates 65-67). Grew's description stands to the present day without modification, except that in the words "from which (the main branches) a few small fibres are dispersed without any order through the apple," "few" should be "numerous." Wortmann, in 1892, specially discussed the relation of the fine bundle endings and branches in the pulp to bitter pit, and Brooks (Bull. Torrey Botanical Club, xxxv. p. 423, 1908) represents the vascular system of the apple, and made dissections of the vascular network from frozen pulp. References to the vascular network in the apple are scattered throughout the literature dealing with the diseases of the apple. Hence it is difficult to understand why McAlpine (Report p. 36), describes the vascular network in the apple pulp as "this wonderful and hitherto unsuspected structure," and when McAlpine states (p. 28), "I venture to think that if these vessels, as well as the wonderful vascular network immediately beneath the skin, where the bitter pit originates had been recognised by Professor Ewart, he would have arrived at a very different conclusion," he makes both a misleading and an unwarranted statement.

A curious error also lies in the statement that "the vascular network is a strengthening system or skeleton," "with a fibrous portion to strengthen the delicate cells and prevent collapse." (p. 36). Fortunately for orchardists, this statement is untrue, since were it true apples would be difficult or impossible to eat raw. Cursory examination of the vascular bundles under the microscope shows the feeble thickening of the wood vessels which are conducting rather than skeletal structures, and the conspicuous absence of true skeletal elements such as wood fibres, sclerenchyma, collenchyma, etc. The rigidity of the apple is due to its distended pulp cells almost entirely, and when these collapse or lose water, the tissue becomes soft and flabby, although the rigidity of the vascular system is unaltered. The latter resembles a capillary blood system, rather than a skeletal framework.

McAlpine's theory of Bitter Pit.

This is in brief (p. 73) that when the supply of water is abundant the vascular network may not enlarge as rapidly as the pulp cells. "a mesh here and there will be left unfinished, the cells adjoining will not receive their regular supplies of nourishment through the regular channels, and collapse and death will ensue."

If the supply of water is deficient, "even if the mesh was completely formed, wherever the mesh of the network of vessels failed in conducting water, there the adjoining cells would collapse, and the entire patch shrivel and become brown."

McAlpine is apparently unaware of the fact that water can pass readily from one pulp cell to another, and that the starch grains usually present in abundance in bitter pit tissue, are carried to the cells through the vascular bundles in the form of sugar, showing that the bundles are functioning normally. It would be easy to obtain evidence of such interruption if it took place. No such evidence is brought forward, and none is to be obtained by the examination of bitter pit tissue. Furthermore, where the bitter pit tissue may form a continuous layer near the surface, as in some confluent forms of "crinkle," living tissue may occur outside the affected portion of the vascular network, from which on McAlpine's theory it should be cut off. His theory is based upon a series of assumptions, some of which are quite incorrect, and no experimental or anatomical evidence is brought forward to support it. The exciting cause demanded is either an excess or a deficiency of water, i.e., diametric opposites producing the same result.

Finally, I have shown that single pulp cells, or small groups of them, in immature bitter pits may retain their starch grains and remain living and turgid until the apple is fully adult, i.e., after the apple has been plucked and the flow of water and food materials through the vascular bundles has ceased. In such cases we are dealing with an inhibition of ferment action by an agency not strong enough to immediately kill the protoplasm, and the death of the starch-containing cells is simply hastened somewhat as compared with sugar-containing cells, possibly partly as the result of starvation. Under normal circumstances the sap of each cell is able to hold all the sugar produced from its starch, and since the protoplasm produces its own diastase, it is impossible to see how an interruption of the vascular system could prevent the conversion of the starch into sugar.

The poisoning theory of Bitter Pit.

A satisfactory theory in regard to a disease or defect should :—

(a) Coincide with all the facts.

(b) The suggested agency should be able to produce the disease or defect experimentally.

(c) The artificially and the naturally produced disease or defect should behave with approximate similarity under corresponding external conditions.

In regard to (a) the poisoning theory agrees with all that we know in regard to the action of ferments, to the sensitivity of the pulp cells to poison, and to the variation of their sensitivity with age, so that an amount of poison at first sufficient to arrest ferment activity would become generally toxic when the protoplasm reached a certain age. In regard to (b) it has been shown that every symptom of bitter pit, including external appearance, colour, character of dead cells and cell walls, and presence of starch grains can be produced by artificial poisoning. The last feature, on which so much stress is usually laid, is a general but not an unavoidable accompaniment of bitter pit. Any agency, including mechanical injury which kills the cells while packed with starch, will cause dead starch-containing tissue to be present in the apple pulp. If, however, the poisoning takes place before the starch has been deposited or after it has been dissolved, the dead bitter pit tissue will not contain any more starch than the living pulp.

In the early proteid stage of the apple, the cells are resistant to poison, and owing to the rapid cell multiplication and proteid growth, no concentration of absorbed traces of poison is possible. In the starch stage the cells are still resistant, and diastatic activity is the first function to be affected. Odd cells here and there are to be found in the pulp of most sound apples in which the starch grains have remained undissolved, but in which the poisoning progresses no further until the general pulp is approaching death. The reason why bitter pit is generally accompanied by cells packed with starch is because it is when proteid growth has ceased, that a concentration of absorbed traces of poison becomes possible, and because the sensitivity to poison increases from this stage onwards in the life of an apple.

It is in regard to the analytical evidence that the greatest difficulty of complete proof is to be expected. The tests carried out in conjunction with the Federal Analyst have shown that it is possible to produce artificial bitter pits with traces of poison so small as to be incapable of detection even by deli-

cate chemical analysis. McAlpine (p. 70 of Report) dismisses the poisoning theory of bitter pit in five lines as follows: "This theory is sufficiently disposed of by the analysis of pitted apples made by Mr. P. R. Scott, Chemist for Agriculture. A State Committee was appointed to collect pitted apples from unsprayed orchards, and on analysis, not the slightest trace could be found of either lead or arsenic, or any other mineral poison." Apart from the fact that the quantity of pitted apples from unsprayed orchards which this Committee, of which I was a member, was able to obtain was exceedingly small, and quite insufficient for an exhaustive examination of the delicacy needed, no attempt was made to detect mineral poisons in general. Mr. Scott practically confined his tests to the detection of arsenic. The only other test used was as follows, "Another portion of the dried material was incinerated at a low heat, and the ash dissolved in hydrochloric acid, and a current of sulphuretted hydrogen was passed through the solution. I did not obtain any coloration of liquid or precipitate by sulphuretted hydrogen." McAlpine is evidently unaware that this method would fail entirely to detect manganese, cobalt, nickel, zinc, aluminium, iron, or chromium. Further it would be entirely useless to attempt to detect lead, mercury, silver, gold ,or copper by this method when present in dilutions just within the toxic limit to the pulp cells of adult apples, without using very large quantities of material and special methods of extraction and mechanical or electrolytic concentration.

Only one experiment is given with a metallic poison—(Report p. 23)—and as an adult apple was used, in which the starch had all or practically all dissolved, little or no starch could be expected to be present in the dead tissue. A saturated solution of mercuric chloride in alcohol was used, which latter is able to kill the pulp cells by merely drawing water from them, and also interferes with the absorption of the mercuric chloride by the pulp. As the solution used had a concentration of 1,000,000,000 times above the toxic limit for Yates' Pippins, it is not surprising that some effects were produced.

In regard to the difficulty raised as to how poisons could be absorbed from the soil by the delicate root-hairs, and the statement that "the concentration within the cells must become more or less equalised with that without, before the plant can be properly nourished," (p. 27, of Report), every student of plant physiology knows that the latter statement is quite misleading. It is also well known that the roots of various plants can absorb traces of various mineral poisons, which may accumulate in special

parts or organs, particularly such as are ultimately thrown off (leaves, bark, fruits), without either the roots or the plant as a whole being affected. The following poisonous metals may be absorbed by various plants when grown on soils containing them: Zinc up to 13 per cent. of ash, manganese up to 14 per cent., cobalt, nickel, mercury, silver, copper up to 1 per cent., lead, thallium, arsenic, titanium, etc. These absorbed poisons are either set aside in special parts or cells sacrificed as poison traps, or may not cause any injury at all if the plant has developed the power of precipitating them in an insoluble or innocuous form.

Actual tests with the roots of seedling apples showed (with sulphate of copper) a toxic limit lying between 1 in 100,000 and 1 in 500,000, although when transpiration is active the limit may be lowered, while when growing in garden soil seedlings may be watered with much higher concentrations without being appreciably affected. In the case of Yates' Pippin the toxic limit of the pulp cells (1 in 1,000,000), is not much lower than that of the roots, which may explain why this variety is practically immune to bitter pit, whereas in the varieties more sensitive to poisoning and to bitter pit, the ultimate toxic limit may be 100 times lower than for the roots, which allows an ample margin for differential poisoning. The pulp cells are end points where poisons may accumulate until a toxic limit is reached, and this toxic limit falls with increasing age or rising temperature. In addition waste and poisonous substances frequently tend to accumulate and concentrate in particular cells or groups of cells which are sacrificed for the benefit of the rest. Evidence has already been brought forward to show that this occurs in the apple.

Some of the experiments brought forward to show the absence of any connection between spraying and bitter pit are worthy of comment (Report p. 23). In a Deepdene orchard, where twelve trees were to have been cut down on account of bitter pit, these were reserved, and left unsprayed. As the result a total of 23¼ lbs. of fruit were formed, including twelve apples affected by bitter pit. So that, apparently, if the experiment indicates anything, it shows that the absence of one year's spraying reduced the bitter pit apples to one per tree!

At Burnley Gardens some fruits were enclosed in bags and others left exposed on an unsprayed tree of Annie Elizabeth. Of the apples enclosed in bags 55 per cent. were pitted, and of the exposed one 39 per cent. Calico bags were used, however, and as calico glazes sometimes contain zinc oxide and other metallic poisons, it would be interesting to know whether the bags were tested before use.

A very important point is to be noted in the Burnley Gardens records given as an appendix, namely, that varieties which in other places are given as immune, or nearly so, to bitter pit, appear to be very liable to it when grown in the Burnley orchard. Thus the degree of affection of certain varieties by bitter pit is as follows :—

	For Orchards generally.	For the Burnley Gardens.
Rome Beauty	Slight to very slight	Bad
Munroes Favorite	Very slight	Slight
London Pippin	Very slight	Bad
Dumelows Seedling	Slight	Bad
Gravenstein	Very slight	Slight
Statesman	Slight	Bad

Now for many years the Burnley Gardens have probably been the most thoroughly sprayed piece of ground in Victoria, and possibly in Australia. In France, it has been found that of the total copper applied as sprays during twenty years, one-half to two-thirds was retained by the soil, and could be recovered on analysis. At the Lausanne Viticultural Station (Switzerland) the surface foot of soil was found to have accumulated 3.5 parts of copper per 100,000 of soil, and in other vineyards as much as 11 parts per 100,000 were found. These figures are well within the limit of toxicity to the pulp cells of apples, even without any accumulation or absorption. It would be of interest to have an analysis of the Burnley orchard soil both now and after an interval of some years.

The British Board of Agriculture (Journal, vol. xix., p. 751, 1912) has recently carried out experiments to determine whether copper which is used in spraying or treating potatoes for various diseases, can be absorbed by the tubers, and to what extent. In the following table of the results obtained the numbers give the grains of copper found per lb. of the dry weight on analysis of the harvested tubers :—

	75 lbs. Strawsonite per acre.		75 lbs. Copper Sulphate per acre.		Untreated.	
	Peel.	Pulp.	Peel.	Pulp.	Peel.	Pulp.
Lancashire plots	1.44	0.10	0.16	0.08	0.12	0.07
Kew plots	0.08	0.045	0.094	0.051	—	—

The highest amounts in the pulp would not be appreciably greater than 1 part of copper per million of the dry weight, and since only living tubers would be harvested and tested, the cells of potatoes seem to be less sensitive to copper than the pulp cells of apples. The "untreated" soil must have contained a fair proportion of copper, and the lesser absorption from the Kew plots is probably

due to the soil containing more humus, which has a pronounced retentive and restraining action upon many mineral poisons, as compared with sandy soils.

The browning of the pulp cells.

According to McAlpine (Report p. 12), the browning of dead pulp tissue is due to a change in the cell walls, and "the gummy or mucilaginous substance which colours them brown is of a pectic character." As a matter of fact the brown colour is not in the cell wall, but in the protoplasm within it; it is not of a pectic character, and it is not either gummy or mucilaginous.

Lindret (Le Cidre, p. 150, 1893) concluded that an oxidase ferment was present which carries oxygen to the tannin of the cell and causes a production of dark coloured oxy-compounds which are precipitated upon the cell walls as a permanent dye. Behrens (Centralbl. f. Bakt. 2 abt. 1898, Bd. 4, S. 514) explained the brown colour as being due to the combination of a direct oxidation product of the tannic acid of the fruit, with the proteids of the cell. Before any definite conclusions can be made, however, it is necessary to know the distribution of the tannic acid in the cell, whether an oxidase ferment is present or necessary, whether a brown colouration can be produced in the absence of free oxygen, and what are the influences of different external conditions on the browning.

The distribution of the tannin in the cell.

Sap rapidly expressed by strong pressure from Statesman, Sturmer Pippin and Yates' apples, and filtered rapidly with the aid of a suction pump in 1-3 minutes, is quite colourless, and remains so on standing. It contains an iron-greening form of tannic acid.

The pressed pulp rapidly browns in air, and with $Fe\,Cl_3$ turns black. Hence one at least of the substances causing browning is not in the cell sap, but in the protoplasm or cell walls of the pulp cells. The black colour with $Fe\,Cl_3$ is produced rapidly with crushed cells, and is darker than before the sap was pressed out, but with living cells it only appears as the $Fe\,Cl_3$ penetrates the protoplasm and kills it. In freshly browned pulp, the colour is not in the cell wall, but in the protoplasm.

The residue of pulp cells on the filter in all cases slowly turns deep brown in air and turns dark with $Fe\,Cl_3$. At first it may contain still living pulp cells, and hence gives the $Fe\,Cl_3$ reaction more rapidly on boiling. Sap filtered from pressed pulp after

standing is distinctly brown, darkens with Fe Cl_3, and shows a faint pinkish shimmer against the brown with solid KCN. With an excess of alcohol and ether a flocculent brown precipitate is formed, soluble in water, and consisting largely of glucose, but blackening with Fe Cl_3 and showing brown with a pinkish shimmer with KCN.

If the slowly expressed sap is boiled before filtering, the filtrate remains almost colourless in air, but turns brown with NaHO, pink with KCN, disappearing and reappearing on shaking and standing, and green darkening to a distinct tannic acid reaction with Fe Cl_3. The residue on the filter paper contains less tannic acid. Apparently some of the tannic acid present in the slowly expressed sap is not present in the natural sap, but is taken up from the protoplasm after the latter has been killed. The non-browning of the sap from boiled pulp suggests the presence of an oxidase enzyme destroyed by heat.

J. af Klercker (Bihang till d. Svenska Vet. Ak. Handl. Bd. xiii. III, 1888), concluded that tannin might either occur in the cell sap or in the protoplasm in the form of oil-like drops formed by the fusion of smaller ones, but that the actual substance of the protoplasm was always free from tannin. To test this apples peeled on one side (Plate V.) were immersed in solutions of ferric chloride, and of methyl blue and the coloured pulp examined after some days. With Fe Cl_3 the walls of the pulp cells remained practically colourless, and also the vacuole, whereas the protoplasmic contents turned brown. Under high powers round or occasionally oval vesicles of tannic acid stained brown with Fe Cl_3, and dark blue with methyl blue, could be distinguished readily in the protoplasm of the pulp cells. Apparently the Fe Cl_3 causes a slight contraction in size of the vacuoles, but in very many cells they are extremely abundant and conspicuous, and none of the pulp cells appear to be entirely free from them. If these tannic acid vacuoles exist in the living protoplasm, they would constitute a second osmotic system, each vacuole being surrounded by either a precipitation membrane or an organised plasmatic membrane, so that tannic acid could only escape from them when the osmotic membrane was destroyed and no new one formed.

The nature of the tannin material.

From its relative solubilities in alcohol and in ether, it may be concluded that it occurs in the form of tannic acid and not of gallic acid. Nevertheless, on boiling acid pulp some of the tannic acid

may be converted into gallic acid. There is no evidence to show that it is present as a glucoside. Since tannin vacuoles must have an osmotic pressure corresponding to that of the cell in which they lie, with their surface tension pressure added (possibly more than 1 atmosphere), the tannin in them must be more concentrated than in the cell-sap.

Exact estimates of the amount of tannin present in the cell are impossible, owing to the difficulty of extracting the whole of it. If any oxidation occurs the brown oxy-tannin combines very firmly with the protoplasm. Although traces of brown colouration may be imparted to the sap at first, once the browning is complete, it is not removed by 1 per cent. hydrochloric or sulphuric acids, darkens in stronger acid or in dilute sodium hydrate, and is not removed by ether, alcohol or peroxide of hydrogen. In a saturated solution of sulphurous acid the pulp rapidly becomes a lighter yellowish brown, but retains this colour even after two weeks without further fading. On washing, the pulp turned to the same shade of black with Fe Cl3 as before. After three weeks in hydrogen peroxide the brown pulp was distinctly paler, and gave a fainter tannin reaction with Fe Cl_3.

Estimations of the tannic acid in a mixed solution are difficult to carry out accurately. In contact with granulated zinc or zinc foil tannic acid is slowly precipitated as a white tannate soluble in HCl, and a slow evolution of hydrogen is shown. With magnesium the evolution of gas is somewhat more rapid, but the white precipitate largely adheres to the magnesium, and becomes browned after a time. The first method, however, can be used even when the tannin is in an acid cell sap, and it appears to be capable of quantitative use, but it is so far not possible to devise a method which will determine the exact amounts of tannic acid originally present in the acid pulp, and apparent differences in the tannin contents of dead and living parts may be merely the result of unequal extraction.

The influence of oxygen on browning.

Preliminary trials showed that both peeled and unpeeled apples could stand repeated evacuations and replacement of an atmosphere of hydrogen or carbon dioxide for three days without the pulp cells being affected. Slices were then floated on glass boats on a one per 1000 solution of copper sulphate, and the air removed by repeated evacuation and replacement with hydrogen or carbon dioxide. They were then shaken into the liquid, and all gas drawn out by evacuation. After one day all the slices were quite unbrowned,

whereas slices floating on copper sulphate exposed to air were browned right through in this time. On admitting air the colourless slices rapidly browned on the surface, and more slowly in the deeper water-logged pulp.

Slices immersed in absolute alcohol slowly browned, especially along the veins, while slices in absolute alcohol under kerosene and in kerosene remained almost entirely colourless after one week, then slowly browning to some extent on exposure to air. In alcohol a little of the tannic acid slowly dissolves out.

The oxidase ferment.

The rapidly filtered sap from apples has no power of decomposing hydrogen peroxide, or of turning guiacum blue. The pounded pulp produces a slow evolution of oxygen gas, and turns guiacum blue, but the same is shown after the pulp has been soaked in 1 per cent. solutions of mercuric chloride or copper sulphate, and then well washed. Boiled pulp causes a feeble decomposition of hydrogen peroxide, such as is produced by various organic materials or finely divided particles.

Pulp cells killed by immersal in 1 per cent and 0—1 per cent. solutions of soluble mercury or copper salts turn brown almost as readily as when killed by crushing. The same is shown when the pulp cells are killed by water saturated with chloroform or with chloroform vapour. In one experiment the presence of chloroform appeared to retard browning Thus a band of skin 2 centimetres broad was removed around the equatorial peripheries of two apples, and one was immersed in (a) a 1 per 1000 solution of copper sulphate, the other in (b) a similar solution saturated with chloroform. In 1 week the brown tissue was 5—6 mm. deep in (b), and 8—9 mm. deep in (a). The central pulp, however, contained much air in (a), but was nearly fully water-logged in (b), apparently as the result of the influence of the chloroform on the surface tension of the air in the intercellular spaces. The different depths of browning were not therefore due to chloroform retarding the browning, but to aeration accelerating it. On exposure to air the central pulp of (b) browned inwards rapidly, but that of (a) more slowly.

If hydrogen peroxide is present tannic acid instead of turning brown with sodium hydrate, gives a light blue rapidly darkening, becoming dirty, and finally brown on standing. Since the pulp cells do not show this intermediate change, but turn directly brown with sodium hydrate, no hydroxyl appears to be present or to take part in the oxidation.

The influence of heat.

Slices of pulp dropped into boiling water until heated through remain colourless in the presence of oxygen, but the pulp gives strong and the colourless liquid faint reactions for tannic acid ($FeCl_3$. KCN, etc.). After boiling filtered apple sap with dilute tannic acid, the liquid turned as black with $Fe\ Cl_3$ as before boiling. A section of pulp browned to half its depth by soaking in dilute tartaric acid and then boiled, did not develop any further brown colouration on exposure to air, but both the pale and the brown parts gave tannic acid reactions ($Fe\ Cl_3$ KCN). Boiled colourless pulp soaked in dilute sodium hydrate or ammonia and exposed to air slowly turns reddish brown by oxidation right through. Hence the absence of browning in boiled pulp is not due to any decomposition or complete removal of the tannic acid in the pulp cells.

If the pulp is slowly heated up to the lethal temperature in air it turns brown, and the same occurs in water, although the browning here is less pronounced, and the water accumulates small amounts of tannic acid.

Before we can understand these results it is necessary to consider the influence of acids on browning, since although solutions of tannic acid rapidly oxidise when alkali is added, they are not appreciably oxidised when directly exposed to air for a week or more either when pure or in the presence of glucose, cane sugar, or citric, tartaric or malic acids, or only acquire a very faint yellowish tinge on very prolonged exposure to air, which necessitates the use of sterilised spore-free solutions.

The influence of acids on the browning of the pulp cells.

When the pulp is immersed in any mineral or organic acid beyond a certain strength, but not strong enough to discolour the cell wall, the pulp cells do not turn brown, but remain colourless, although they are killed. At lower dilutions still sufficient to kill the pulp cells, browning takes place.

Sturmer Pippin Apples.	Concentrations at which pulp cells:—		
	Turn dark brown.	Pale brown.	Remain colourless.
	p.c.	p.c.	p.c.
Sulphuric acid - -	0.01	0.1	1
Oxalic acid - - -	0.1	0.5	1
Citric acid - - -	1	4	7.5
Tartaric acid - - -	1	5	10

The colourless pulp, however, after washing, gave black with $FeCl_3$, and brown with sodium hydrate, showing that the tannic acid had not been destroyed. Colourless acid pulp or colourless boiled pulp turned brown on soaking in dilute sodium hydrate or sodum carbonate or ammonia, but remained practically colourless after pounding up with precipitated chalk.

If the pulp browned by alkali is immediately replaced in acid it becomes slowly colourless again, but if kept for some time the brown colour is permanent, and is not removed by acid. Colourless pulp just neutralised with sodium hydrate, ammonia or precipitated chalk, darkens slightly on long exposure to air, but the colouration is feeble as compared with that produced in the presence of a slight excess of alkali.

Causes of browning. General conclusions.

The browning is due to the oxidation of tannic acid present in numerous minute vacuoles in the protoplasm of the pulp cells, and of an iron-greening tannin present in the cell-sap. The former appears to be gallotannic acid, and in the presence of free alkali its oxidation is not necessarily dependent upon the presence of any oxidase ferment. Oxidase action does not take place in acid media beyond a certain strength. When the protoplasm dies slowly in air the tannic acid in the presence of neutral or alkaline bases is oxidised. If killed rapidly by boiling, the oxidase enzyme is destroyed, and the acid in the cell sap penetrates the protoplasm and removes its alkalinity before the tannic acid has time to be oxidised. On the addition of alkali, oxidation takes place. When the pulp is killed by pounding or by pressure, brown oxidation products may appear in the sap. Tannic acid and the brown oxidation products combine rapidly with dying but uncoagulated protoplasm, and more slowly with coagulated protoplasm. The brown colour imparted to the protoplasm is then very permanent, and is not removed by acid.

When living pulp cells are placed in poisonous solutions which destroy oxidases, the protoplasm is killed, and the tannic acid in the protoplasmic vacuoles is oxidised in the presence of its alkali or alkaline bases and of oxygen before the vacuolar membrane loses its osmotic properties and allows the acid of the sap to penetrate the protoplasm and remove its alkalinity. If the poison used is a free acid above a certain concentration, dependent upon its rate of diffusion, combining avidity and ionisation, the alkalinity of the protoplasm is neutralised as it is killed, and no browning takes place. The tannic acid is, how-

ever, still present in combination with the coagulated proteid, and will give the characteristic reactions and turn brown on adding alkali.

If all the oxygen is removed, the cells can be killed by poison without turning brown, and if the acid sap has been given time to penetrate the protoplasm completely no browning takes place on admitting air until alkali is added.

When slices of pulp are dropped into absolute alcohol, which destroys the oxidase, they remain practically colourless, because the rapid penetration of the alcohol destroys the vacuolar membrane and allows the alkalinity of the protoplasm to be neutralised by the acid sap before the tannin has time to oxidise. Along the veins, however, where there is little or no acid sap, browning may take place, although the oxidase is destroyed by the alcohol.

When an apple is cut with a sharp razor the cut surface remains quite pale, whereas when scraped or cut with a blunt knife it turns rapidly brown.

If the cut or scraped surface of the pulp is moistened with hydrochloric acid of acidity somewhat greater than that of the cell sap (0.5 to 1 per cent.), which diffuses rapidly through the ectoplasmic membrane, the cut or bruised surface remains quite pale.

The relative rates of death, of penetration of acid, and of escape of sap appear to be factors in producing these differences, but the matter needs further investigation.

The Anaerobiosis of the Apple.

McAlpine (Report p. 42) states that when the supply of free oxygen is cut off apples and pears can still live for months. No experiments or references are given in support of the statement. It has long been known that apples will live for a long time in a confined space, but then they contain a large amount of air to begin with, and their respiration is not very active, particularly at low temperatures.

Yates' Pippin apples were placed in an air-tight receiver, exhausted, filled with pure CO_2, again exhausted, and the process repeated several times daily for the first three days, and then every third day. The temperature averaged 14—18 deg. C., reaching 20 deg. C. twice during the longest period of exposure. After two weeks in CO_2 all were sound and living, but after one week in air slight decay was shown at some points near the surface.

After three weeks in CO_2, the pulp was collapsed, and dead on the surface at some points. The fact that the dead tissue was

slightly browned would show that a trace of free oxygen was still present in the pulp.

After four weeks the apples were collapsed, and the surface wrinkled for the most part, the pulp dead and slightly browned, becoming very dark on exposure to air. Portions of living pulp averaging one-fourth of the bulk in some parts extended from the surface to the core.

After six weeks in CO_2, all were dead, the first portions dying being distinctly brown, the later portions to die being practically uncoloured until air was admitted.

Evidently the air is only slowly removed from an unpeeled apple in an atmosphere of carbon dioxide. Using hydrogen, similar results were obtained, but death took place in four to five weeks, probably owing to the more rapidly diffusing hydrogen removing the air from the apple more rapidly.

Apparently a Yates' apple is not capable of more than a month's strict anaerobiosis, and carbon dioxide has no poisonous action on the pulp cells, acting simply by replacing air.

Experiments were also tried on the effects of covering the skin of apples with comparatively impermeable films, and of immersing them in liquids in which oxygen is more or less soluble than it is in water. Of these the results obtained under kerosene and with gelatine films are of most interest.

Apples immersed in kerosene.

Peeled apples under a depth of two to four inches of kerosene remained a pale greenish colour for fourteen days, and the pulp cells were turgid and living. In three weeks they turned soft, and slowly acquired a brownish colour, duller and not so dark as in air. From the surfaces small plasmodium-like masses of granular whitish material exuded, which later appeared to grow over the whole surface as a white felt-like moss. This consisted of very fine, much-branched threads, with no spores and few or no transverse partitions. The appearance was as though a granular plasmodium had turned into a filamentous mycelium.

Slices of raw potato infected with the mycelium developed under kerosene white sunken patches at each point of infection, ramifying at first in the substratum, but later more on the surface, the threads being somewhat coarser, and more septate than on the apple, possibly as the result of better nourishment. Over the uninfected surface and on uninfected slices, small whitish granular plasmodium-like exudates appeared, turning later brownish. These

are merely soluble exudates from the cell precipitated in contact with the kerosene.

Mycelium infected slices of potato and apple in air developed blue patches at each point of infection and characteristic Penicillium sporophores. The same formed slowly on pieces of the original felt-covered apples when kept in moist air. Penicillium is hence able to slowly develop a vegetative mycelium when immersed under a depth of two to four inches of kerosene. It appears also that neither the living ectoplasmic membrane of the pulp cells of apples or that of Penicillium is permeable to kerosene. Chudjakow (Lafar Technische Mykologie, 1, 315) has shown that Penicillium can grow at a pressure of 10 mm. when well nourished (glucose, etc.), and oxygen is about 5 times more soluble in kerosene than in water.

The influence of gelatine skins on apples.

Sound Yates' Pippins which had been kept in cool storage from March 1 until September 1 were exposed to air on a laboratory table, the temperature ranging from 13 deg. C. to 33 deg. C. during the following six months. One half were untreated, the others were momentarily immersed in melted 10 per cent. gelatine at 40 deg. C., and hung up by the stalks to drain. The gelatine soon dried, forming a very thin skin on the surface, and giving the apples a very glossy bright colour. In spite of the blocking of the breathing pores, sufficient air diffuses in to prevent asphyxiation. After two months the untreated apples were darker coloured, more or less pitted, soft or wrinkled, and in two to three months had begun to rot or were completely rotted, in every case the rotted pulp containing fungal hyphae. The gelatined apples after three months were brightly coloured and though slightly soft on the surface were smooth or only slightly wrinkled. In the latter case the gelatine films separated slightly from the skin at one or two points, but no signs of decay were shown. After five months the gelatined apples were still sound, and brightly coloured, but slightly soft or wrinkled in parts, whereas the untreated apples had contracted to shrivelled brown masses. After six months the gelatined apples were still of a sound bright colour, and the flesh of a good flavour, but not quite firm. As the room was a very dry one, the conditions as regards the loss of water were, however, very severe, and the apples had already been six months in cool storage. This simple method of gelatining the skin seems suitable for preparing apples for exhibition or for preserving small lots of valuable apples without the necessity of cool storage. The apples must of course be kept

dry, and should not be in contact, or if so, should be wrapped in tissue paper, and a free circulation of dry air allowed between them. Whether the method would be of any use for extensive storage could only be told by actual trial.[1] The gelatining does not at first affect the flavour of the apples, and if properly done the gelatine skin is hardly noticed when the apple is peeled.

The rotting of apples in storage appears to be almost entirely due to the development of fungal hyphae in the pulp, the spores entering from the surface through the breathing pores of the skin. Gelatining the apple would prevent any entry of spores and remove this source of decay, if no spores had already gained entry. Apart from this source of death, there is no reason why sound apples should not gradually shrivel when kept without any actual rotting occurring. It has already been stated in Germany that apples keep much better if the surface is sterilised by washing in formalin. Wrapping in tissue paper would then prevent the entry of fresh spores from outside.

The drying and contraction of Bitter Pit tissue.

This is the natural result of the fact that the plasmatic membranes on death lose their power of preventing the escape of the dissolved materials present in the vacuole. As these diffuse outwards the water follows them, and is drawn into those still living cells whose osmotic pressure is not fully satisfied. This is aided by the elastic contraction of the previously distended cell walls, and their ultimate complete collapse is hastened by the loss of water by evaporation. With artificial poisoning sunken pits only develop when the poisoning is localised. When the whole surface is browned it remains smooth until much moisture has been lost. The stretched epidermis contracts at first *pari passu* with the collapse of the superficial pulp cells, and it is only when the contraction is excessive that either cracks appear or that the epidermis becomes wrinkled so as to retain the same total surface area.

In the following experiment peeled Yates' Pippins of exactly equal weight (60 grams.) were floated on 250 cc. of each liquid and weighed daily. In the two poisonous solutions the total weights of mercuric chloride present were 0.20 and 0.125 of a gram respectively, amounts too small to affect the weights appreciably by absorption.

1 Mr. Schoubridge informs me that unpublished experiments have shown that in time the flavour is sufficiently affected to lower the saleable quality particularly for export, but no data are available of the varieties tested or of the conditions under which the tests were made.

	Increase of Weight. After 1 day.	During 2nd day.	During 3rd day.	Condition.
	p.c	p.c.	p.c.	
Water	12.8	4.9	4.5	Brown on surface and water-logged to 11mm. depth.
$HgCl_2$. 1 per 10,000	13.9	6.9	2.9	Browned 11mm. deep and water-logged 12m.m. deep.
$HgCl_2$. 1 per 2000	15.1	6.9	4.6	Browned to depth of 14m.m. and water-logged 15mm. deep.

On the third day the apple in the 1 per 2000 solution sank, that in 1 per 10,000 was just afloat, and the one in water, though still afloat, was floating lower than before. The increase of weight results mainly from the water filling the air spaces in the pulp, and the collapse or contraction of the cells killed by poison enlarges the air spaces, and hence accelerates the entry of water.

The distribution of water and ash in apples.

Zschokke (Landw. Jahrb. d. Schweiz, 11, p. 192, 1897), showed that in transpiring apples there might be a difference of from $\frac{1}{2}$ to 1 per cent. in the amount of water in the basal and distal portions of the fruit. This is the natural result of the greater abundance of breathing pores, through which water vapour escapes, on the calyx half of the apple. McAlpine (Report p. 73) gives similar data for Annie Elizabeth apples.

	Freshly Plucked. Top.	Middle.	Bottom.	After 8 days. Top.	Middle.	Bottom.
	p.c.	p.c.	p.c.	p.c.	p.c.	p.c.
Clean	85.50	86.23	86.12	85.68	86.74	86.92
Pitted	85.49	87.04	87.58	86.13	86.51	86.17

Hence the average percentage of moisture in the clean, freshly-plucked apples was 85.95 per cent., and after eight days in air was 86.45 per cent., the numbers for the pitted apples being 86.7 per cent., and 86.27 per cent. respectively. Apparently the percentage of moisture increased in the clean apples after eight days in air, and decreased in the pitted apples, a remarkable result, if not due to faulty methods or the use of unequal material. In any case it is evident that variations of $\frac{1}{2}$ to 1 per cent. of water can be of no importance in regard to bitter pit, for much greater variations are shown by growing and adult apples, and the weight of a fresh apple can be reduced by drying by 5 to 10 per cent. without the pulp cells being injured.

Much greater importance is to be attached to variations in the percentage of ash, although in the case of a strong metallic poison an apple might be completely killed without its precentage of ash being appreciably increased. Mr. P. R. Scott's analyses, however, seem to show that bitter pit apples and pears contain a greater percentage of ash than usual. (Report pp. 46, 47.)

	Percentages of Ash. Sound.	Pitted.
Josephine Pears -	0.38 -	0.43
Stone Pippin Apples -	0.42 -	0.48
Lord Wolseley Apples -	0.271 -	0.352

The pitted fruits only contained 1 to 2 per cent. less moisture than the clean ones, so that the bitter pit tissue must have been in an early sappy stage, and the ash which represents materials drawn from the soil does actually appear to be more abundant in bitter pit tissue or in pitted fruits. No analyses of the ash are given, unfortunately, so that it is uncertain whether the increased ash contains unusual or poisonous constituents.

Effect of manuring on Bitter Pit.

The results of a variety of tests with different manures are given in the Bitter Pit Report in great detail (pp. 80-91). In the Box Hill orchard the percentage of bitter pit in the two check plots was 1.3 and 4.5. The percentages on the manured plots were all less than the difference between these two. In the Bathurst plots the averages for the two unmanured plots were 1.42 and 3.00, and this was a greater range of variation than was shown by any of the manured plots. In the Blackwood orchards the number of pitted apples varied from 1 to 5, and the number of apples from the three controls varied from 1 to 56. None of these tests therefore show anything, the differences observed being of the field variation character.

Effect of pruning on Bitter Pit.

In regard to the results given with pruning (Report p. 92, 98), a similar criticism holds. In the Burnley results, with leader pruning, the bitter pit apples varied from 2 to 21, a greater range of variation than between the different modes of pruning, showing that the numbers given are meaningless as regards the effects of pruning. In the Deepdene orchard the bitter pit apples from unsprayed trees pruned in four different ways totalled only 12, with a variation of 1 to 5. The Bathurst tests are most satisfac-

tory, and in spite of the high range of variation (0.54 to 8.41) seem to indicate that medium pruning produces the largest amount of bitter pit, and no pruning the least, whilst hard pruning is intermediate. Nevertheless on page 81 of the Report medium pruning is recommended as one means of reducing bitter pit. Medium pruning tends to concentrate the sap on the fruits, and with it any poisonous ingredients absorbed, while hard pruning tends towards new wood formation, and so diverts some of the sap from the fruits.

The interesting experiments with different stocks carried out by Mr. Quinn are not yet far enough advanced to enable any conclusions to be made, but grafting is not likely to affect the resistance of the pulp cells of the grafted scions to poisons.

The influence of cool storage on Bitter Pit.

Since the resistance of the pulp cells to poison is greatly increased at low temperatures, it is only to be expected that in cool storage the development of bitter pit should be retarded or even stopped, when only minimal amounts of poison are present. If the starch grains were undissolved, the low temperature would prevent or delay the last stage of complete poisoning and turning brown. If the amount of poison present is relatively large, brown spots may still appear in cool storage, but the amount will be reduced. (Report pp. 103, 105.) The influence of cool storage is mainly confined to preventing or delaying the onset of the later stages of poisoning, such as death and collapse of the cells, the turning brown and acquiring a more or less distinct bitter taste. It is, however, precisely these changes which render affected apples unsaleable. An apple in the incipient stages of bitter pit, though a little less sweet, will be as edible as one not affected at all, and the point may be once again emphasised that an apple may be completely poisoned with various metallic poisons and yet contain insufficient poison to produce any poisoning symptoms or effects when eaten.

Conclusions.

The evidence in favour of the poisoning theory of bitter pit brought forward in the present and previous paper, may be briefly recapitulated as follows :—

It is possible by applying poison during the starch stage of an apple to reproduce artificially every symptom of bitter pit. The diastase ferment which is responsible for the normal solution of

the starch grains is present during at least the early stages of bitter pit. The starch grains are normal and capable of solution. The only agency capable of preventing their solution in localised living cells under the conditions existing is a poison.

The most resistant apple (Yates' Pippin) to poison is also the most resistant to bitter pit.

At low temperatures the resistance to poison is increased ten to a hundred times (0 deg. C. as compared with 30 deg. C.). In cool storage the resistance to the full development of bitter pit is similarly increased.

The poisoning theory is in accordance with all that is known in regard to the sensitivity of the pulp cells to poisons, to their diminishing resistance with increasing age, and to the changes which take place in the cell. The increased percentage of ash in bitter tissue is evidence pointing in the same direction.

Dr. White, in bitter pit apples from an orchard heavily sprayed with arsenate of lead, was able to detect the presence of traces of lead. Against this is the fact that the State Analyst was unable to detect arsenic in a limited number of bitter pit apples from four orchards certified to have been unsprayed. Any poison is, however, capable of producing bitter pit symptoms, and the results obtained by the Federal Analyst show that it is possible to poison the pulp cells of apples by traces of poison so minute as to be incapable of detection even by delicate chemical analysis.

No other theory of bitter pit will stand critical examination or scientific testing. Mr. McAlpine's vascular interruption theory is unsupported by observation or experiment, and is negatived by the fact that during the early stages of bitter pit the vascular connections are normal. The cracks appearing in the tissue form during the later stages, they are not always present, and are the result and not the cause of the death and contraction of the pulp cells. In addition, the starch grains of bitter pit tissue and its ash constituents are carried to it by materials conveyed along the vascular bundles, and since the percentage of ash in bitter pit tissue is higher than in healthy pulp, the conducting channels must have been functioning more actively if anything than usual. The accumulation of absorbed poisons at certain points causes these cells to be sacrificed to maintain life in the rest.

The browning of apple pulp is due to the oxidation of tannic acid. Apparently gallotannic acid occurs in numerous minute rounded vacuoles in the protoplasm of the pulp cells in addition to the iron-greening tannin of the cell-sap. If so, the plasmatic membranes of these vacuoles must be impermeable to alkali or oxygen, or both when

living. The vacuolar membrance is impermeable to the acids of the cell sap. When a metallic poison which destroys oxidase is applied externally, alkali and oxygen may come into contact with the tannic acid and produce browning, before the vacuolar membrane becomes permeable to the acid of the cell sap. When the cells are killed by boiling oxidase is destroyed, and the vacuolar membrane allows acid to escape immediately, and neutralise the alkalinity of the protoplasm, so that the pulp remains colourless until alkali is added. When the cells are placed in toxic but very dilute sulphuric acid, browning occurs before the alkalinity of the protoplasm is removed, or before the oxidase is destroyed. With stronger acid solutions the oxidase is destroyed or the alkalinity is removed before the tannic acid has time to oxidise, and no browning takes place. The brown oxidation products rapidly unite with the proteids of the cell, and the colour is then very permanent. Carbon dioxide and unaltered cell sap are non-poisonous to the pulp cells. Tannic acid is less poisonous than any other acid tried, and alcohol is less poisonous than lime water.

Yates' apples do not appear to be capable of more than a month's strict anaerobiosis in hydrogen or carbon dioxide at room temperature. In a cool chamber the period would probably be prolonged. Apples can be preserved to a remarkable extent by coating them with a gelatine skin. Peeled apples will remain living for a fortnight longer under kerosene, and the fungus Pencillium will grow upon them and upon potato slices submerged in kerosene.

DESCRIPTION OF PLATES.

Plate III.—Localised poisoning, with 1 per 1,000,000,000 of mercuric chloride.

IV.—Fig. 1. Portion of vascular system of apple x 12¾.
Fig. 2. Portion of vascular system of apple x 29.
Fig. 3. Endings of vascular bundles in apple pulp x 22.

V.—Fig. 1. Apples peeled on one side and blackened with ferric chloride.
Fig. 2. Portions of blackened pulp cells x 10, showing tannic acid vacuoles.
Fig. 3. Tannic Acid vacuoles in protoplasm of pulp cells stained with ferric chloride x 320.
Fig. 4. Do., stained with methyl blue x 400.